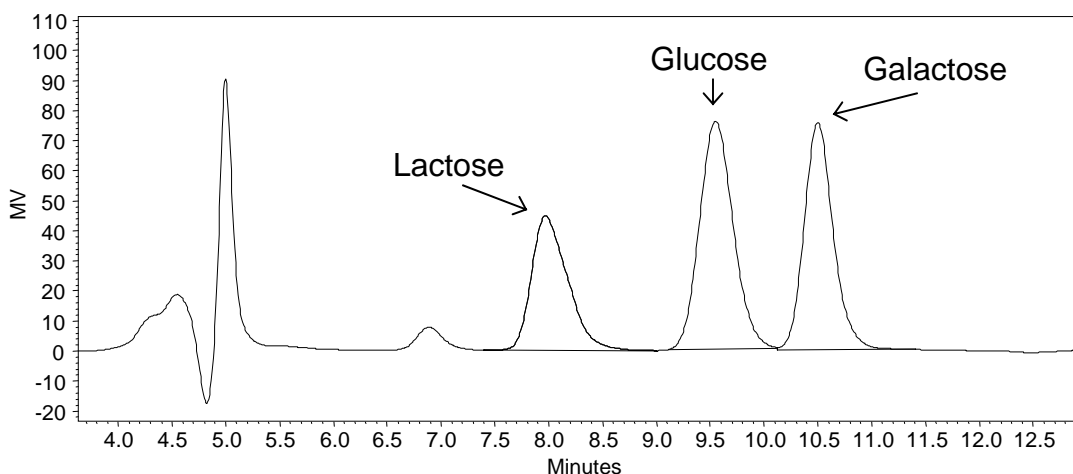


Waters® 2414 Differential Refractive Index (dRI) Detector: Analysis of Milk Sugars

Introduction

The analysis of sugars is a common assay in many food laboratories. Particularly important is the analysis of the lactose content in milk. An estimated 50 million American adults are lactose intolerant, or have the inability to digest significant amounts of lactose, the predominant sugar in milk. As a result, lactose-reduced milk (lactose contained in the milk is reduced by the enzyme lactase into glucose and galactose) is available for purchase in most supermarkets. This Performance PerSPECTive describes the analysis of lactose-reduced milk for the sugars lactose, glucose and galactose.

Figure 1. Separation of Lactose-Reduce Milk (5 μ L injection volume)



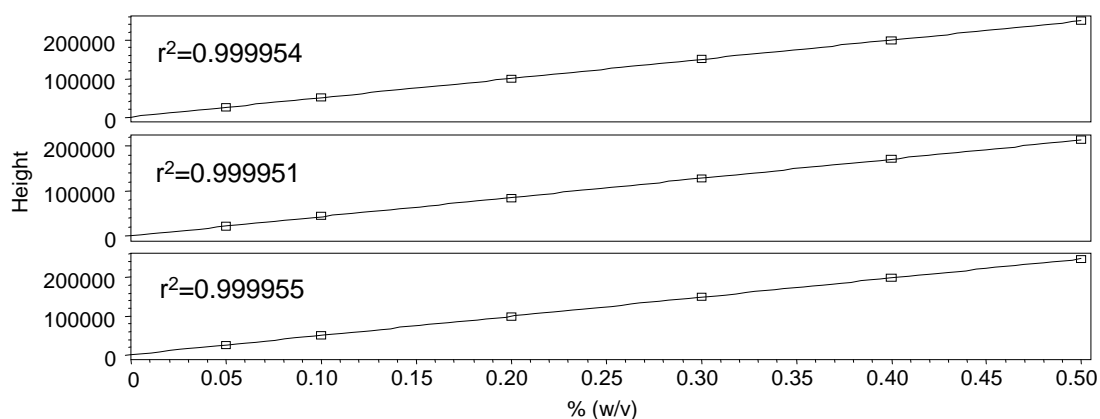
Experiment

Samples of commercially available lactose-reduced milk were prepared by precipitation with isopropanol (1 mL milk + 500 μ L IPA). Following centrifugation, the supernatant was diluted 1:10 with water. Separations were performed with Waters Sugar-Pak™ I column (p/n WAT085188) at a flow of 0.5 mL / min (HPLC grade water containing 50 ppm Ca-EDTA) using Waters Alliance® System (2695 Separations Module). Additional system components included Waters 2414 dRI (Temp. 50°C, Sensitivity 128) and Waters Column Heater Module set to 90° C. A typical chromatogram of a reduced lactose milk sample is shown in Figure 1. Data was collected and analyzed using Waters Empower™ Software.

Calibration

Standard solutions of lactose, glucose, and galactose were prepared in water at concentrations ranging from 0.05 – 0.50% (w/v). Calibration curves for all three compounds were linear (r^2 values greater than 0.9999) as shown in Figure 2.

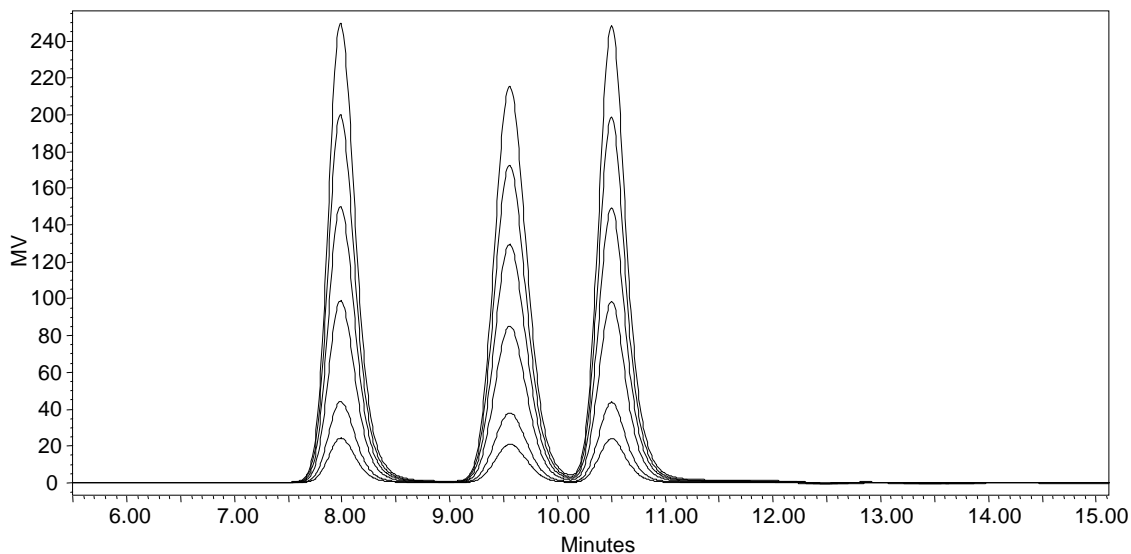
Figure 2. Calibration Curves for Lactose, Glucose and Galactose



Results

Retention times for lactose, glucose and galactose were 7.98, 9.55, and 10.50 minutes respectively, and were extremely reproducible as seen in Figure 3 (i.e., retention time deviations were less than 0.004 minutes for each compound). Quantitation was performed using an external standard method based on peak height. Samples of reduced lactose milk contained on average, 1.36% lactose, 2.70% glucose, and 2.31% galactose. The amount of lactose found in the assay was consistent with the label claim of a 70% reduction in lactose compared to regular milk.

Figure 3. Overlay of Standards 0.05% - 0.5%



Summary

- The analysis of sugars in milk products can be reliably and reproducibly performed using the HPLC method described in this PerSPECTive.
- Waters 2414 Differential Refractive Index detector combined with a Waters Alliance® HPLC System provides superior stability, linearity, and reproducibility required for today's laboratories.